Modern aspects of peptide immuno-modulating agent use in the system of vaccinal prevention and vaccinal therapy.

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The use of peptide immuno-modulating agents for vaccinal prevention in population does not have practical application for a number of reasons. At the first place it is related to the fact that occurence of postprimary immunodeficient condition in subjects who must be vaccinated normally does not lead to significant decrease of spesific immune response and is not considered to be contradiction to vaccination. Therefore, perspective of immuno-modeling agent use in the system of vaccinal prevention can be based on the use of such agents as medicamentous «cover» for post-vaccinal period to decrease undesired viccanal reactions, prevention of some post-vaccinal complications, reduction of number of contradictions to vaccination among specific groups of people with some chronic conditions, or to stimulate earlier protective immune response among risk groups: children with hemoblastosis when immediate and regular blood transfusion is needed, newborns of viral hepatitis B mothers, and generally healthy people in need of immediate vaccinal prevention in site of infection.

Among the most important reqirements for such agents is the ability to stimulate formation of certain classes of specific immunoglobulins, regulate production of immune and inflamation mediators and also regulate oxidative stress reaction in situaion of massive dose antigen input.

It is known that progression of inflamation reaction is realized by increase of inflamation pro-mediators production which, in codition of healthy activity, are present in the body in physiological concentrations and are responsible for function regulation on cell and tissue levels. Some of them such as TNF, IL-1, IL-4 or IL-6 are also the mediators of immune system cells, when others like active oxygen metabolies provide healthy functioning of oxygen-dependent neutrophil bactericidal action system.

Massive impact of antigen irritator of different ethiology induces oxidative stress processes and insrease of production of mentioned above immune mediators which can in this case cause demage and are the reason for local inflamation site development or system disregulations of different organs and tissues, allergization, lipid peroxidation increase and development of intoxification and febrilly symptoms. With this in mind

the use of peptides with regulatory activity that provide regulation of above-noted parameters among subjects predisposed to hyperactive reactions and among subjects with chronic conditions makes it possible to improve vaccine safety and reduce its' undesired side-effects.

Clinical study of new pharmaceutical immuno-modeling drug Imunofan that contains synthetic regulatory peptide has demonstrated its ability to activate antioxidative protection system and regulate formation of inflamation intermediates in patients with severe cronic diseases [3]. For instance, administration of the drug in cases of lymphoproliferative diseases and chronic viral infections promotes lipid peroxidation decrease, inflamation promediators formation, reduction of intoxification and febrilly symptoms. No side-effects were noted upon administration of the drug by adults and children [4].

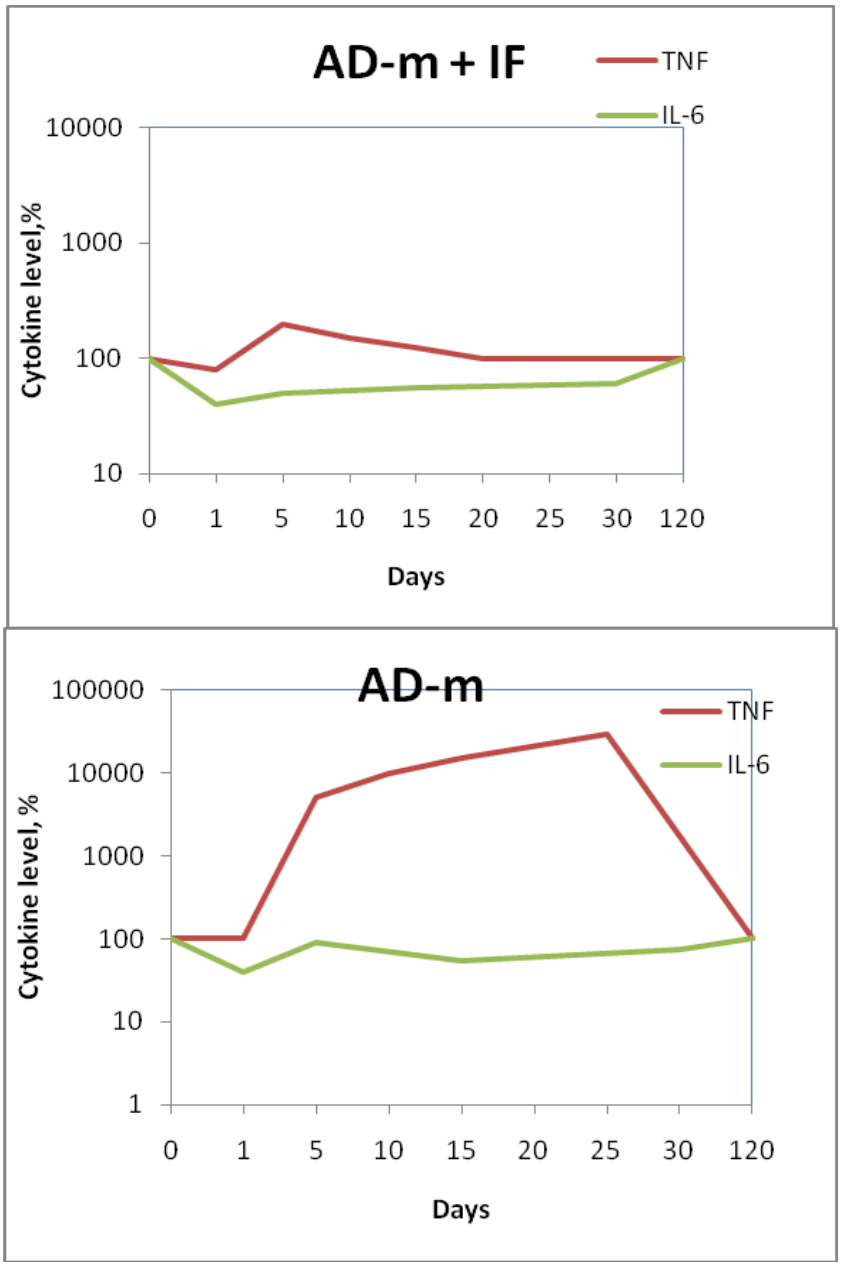
Thus, the results of clinical study of Imunofan with participation of chronically ill patients has demonstrated its ability to regulate production of inflamation mediators and activate antioxodative protection system what is considered to be of an interest for further evaluation of the drug impact on mechanisms of inflamation reaction progression and specific immunoglobulin production upon vaccine injection.

With this reason the dynamics of TNF production - key inflamation mediator and IL-6 - B-cell differentiation factor was evaluated in control group of generally healthy subjects immunized with vaccine with reduced level of AD-m-anatoxin antigen. Single injection of vaccine to adults induced significant increase of plasma TNF levels starting on days 3-4 of the onservation period. Further increase of TNF production was observed for the period of 10-12 days after immunization when its plasma concentration went up to 1000 pg/mcl what estimates approximately 10-fold increase of physiological normal level. This marginal increase of inflamation promediator production corresponds with levels in patients with septic condition or with acute phase of viral disease. Dynamic changes of B-lymphocyte differentiation factor (IL-6) production in vaccinated subjects were demonstrated by moderate supression, however, that was not considered statistically reliable (diagram 1).

Thus, injection of the least reactogenic anatoxin AD-m induces acute increase of the key inflamation mediator - TNF production and, as result, disruption of cytokine immune and inflamation reaction cascade. Above-noted disruptions have transient nature, however, their measure indicators correspond with those in patients with suppurative-septic complications or oncologic pathology.

Single injection of 1,0 ml of Imunofan in combination with AD-m vaccine to subjects in clinical study group helped to maintain physiological levels of TNF- and IL- 6 production throughout the whole period of postvaccinal observation (diagram 1).

Diagram 1. Production of pro-inflamation cytokines TNF and IL-6 after injection of AD-m or AD-m with Imunofan to healthy donors. Axis of abscissa: time period after vaccine injection; axis of ordinates: cytokine level in %% to pre-vaccination levles * - $p<0,05$ compared to initial levels.



Thus, the use of peptide immuno-regulatory drug Imunofan in combination with vaccine allows to prevent hyperproduction of key inflamation mediator TNF and to decrease the disruption of immune cytokine reaction cascade during post-vaccinal period.

Correction of post-vaccinal cytokine disruption demostrates the ability of peptide immuno-regulatory drug not just to make positive impact on pathogenetic mechanisms of reactogenic reaction induction but also it assumes the ability to change levels of certain classes immunoglobuline production and potency of immune response.

With the purpose to evaluate perpectives of Imunofan administration as adjuvant vaccination agent it was considered to be of an interest to study the impact of Imunofan on production of specific antibodies in clinical trial with certain vaccines including cultural antirabbies vaccine, vaccine against infectious virus rhinotracheitis - infectious pustular vulvovaginitis and also vaccines against tick-borne encephalitis and hepatitis A.

Experimental study of Imunofan impact on infectious and vaccinal process.

Immunoregulatory impact of Imunofan on cellular and humoral immune indicants intends its ability to improve specific immune response to viral antigens and provide protective effect in case of viral inoculation. It seemed to be of an interest to study an impact of Imunofan on immunogenic activity of some antiviral vaccines, its protective effect on body upon virus attack and survivability of an offspring in case of embryonic infection.

Impact of Imunofan on production of virus-neutralizing antibodies upon immunization with infectious virus rhinotracheitis - infectious pustular vulvovaginitis vaccine (IRH-IPV)

During the works on development of inactevated vaccine for IRH-IPV for the cattle (done by Sverdlovskiy **Research Institute**) experimental series was developed, however it did not show immunizing power high enough so that further work has been conducted to improve vaccine immunogenic activity by addition of immunomodulatory medication Imunofan [33] to vaccination protocol. An impact of medication on the strength of vaccine for IRH-IPV in oxes of Sverdlovskiy breading assosiation is shown on Diagram **2**.

Use of single vaccine induces production of virus-neutralizing antibodies in 1:4 - 1:16 titer after 30 days since immunization. However, by day 90 since immunization specific antibody titer drops down to 0 - 1 ;4. One-time injection of Imunofan the day before vaccination in the dose of 1 mkg/kg per body weight induces increase of neutralization reaction indicant at the presence of virus IRH-IPV antibodies up to level of 1:32-1:128 after 30 days. Also high levels of protective antibody titers were observed over the whole trial period.

Thereby addition of Imunofan to immunization protocol allows to significantly increase level and term of virus-neutralizing antibody circulation.

Impact of Imunofan on immunogenicity of tick-borne encephalitis vaccine.

Trial was conducted with the use of Balb/c mice with 12-14 g body weight. Branch standard norma (BSN) of tick-borne encephalitis vaccine (cultural, inactivated, fiber-entrapped, fluidus) VKE 201 series developed by Institute of polyomyelitis and encephalitis of Russian Academy of Medical Science (RAMI) of Moscow was used for vaccination

VKE dilutions of 1:10, 1:32, 1:100, 1:320 were subdermally injected in the volume of 0,5 ml. Imunofan was subdermally injected in the dose of 0,05 mkg per one mouse in the volume of 0,3 ml. Vaccine and Immunofan were injected both at once three times with one day stretch. Each dilution was injected to 10-12 mice. Tick-borne encephalitis of Absettarov strain was injected intraperitonially in the volume of 0,25 ml on the Day 9 after the end of immunization. Inoculating dose of tick-borne encephalitis virus was 320 LD_{50}

Mice were observed for 14 days after tick-borne encephalitis virus inoculation.

Trial results were evaluated by 3 indicants:

Rate of survived animals per each vaccine dilution

Value of maximum allowable dilution that provided 50% mice survival rate after tick-borne encephalitis virus inoculation estimated according to Reed-Muench (PR_{50}) formula

Value of minimum immunizing dose of VKE that protects 50% animals (MID_{50}).

Table 2. Impact of Imunofan on immunogenicity of tick-borne encephalitis vaccine

Animal group	Vaccine dilution	% of survived animals	PR_{50}	MID_{50}
Control group (VKE)	1:10 1:32 1:100 1:320	80 60 20 0	1:38	0,013
VKE + Imunofan	1:10 1:32 1:100 1:320	100 63,6 50 0	1:72	0,007

As the Table 2 shows, with 1:10 vaccine dilution all mice received both vaccine and Imunofan have survived, at the same time among mice received vaccine only survival rate was 80%. In the dilution of 1:100 vaccine protected 20% mice, vaccine together with Imunofan showed to be 2,5-fold more effective and protected 50% animals. With vaccine dilution of 1:32 and 1:320 impact of Imunofan on VKE immunogenicity was not revealed. PR_{50}value increased 2-fold, MID_{50} value decreased 2-fold , i.e. to protect 50% animals in case of combination of vaccine and Imunofan it is possible to use 2-fold more diluted vaccine (value PR_{50}) or of the same vaccine concentration but 2-fold lower dose (value MID_{50}) compared to the group without Imunofan [15,28].

Thereby addition of Imunofan to tick-borne encephalitis vaccine immunization protocol allows to significantly increase its immunogenicity and animal survival rate after virus inoculation.

Experimental study of Imunofan impact on infectious and vaccinal process in cases of rabies

Trial was conducted with the use of white outbread mice of 12-14 g body weight. Cultural antirabies vaccine (CAV) c. 117 produced by Institute of poliomielitis and viral encephalitis of Russian Academy of Medicine (RAMI) of Moscow was used. Inoculation of mice was done with fixed rabies virus (SU8 strain) by intracerebral injection of 0,03 ml on the Day 8 past immunization, operating dilution was 3-3,6 $lgLD_{50}$. Imunofan in the dose of 0,05 mkg in the volume of 0,5 ml was injected subdermally right after mice inoculation with rabies virus. At the same time with inoculation and Imunofan injection, first subdermal immunization with 0,1 ml of antirabies vaccine was performed. Undiluted

CAV and dilutions of 1/4. 1/16, 1/32, 1/64 were used for vaccination. Each dilution was injected to 14 mice. Imunofan and vaccine were injected to mice 5 times for 5 days. There were 2 control groups of mice with rabies virus: one group received vaccine without Imunofan and another group receievd Imunofan without vaccine. Mice were observed for 14 days after rabies virus inoculation. Results of the trial were evaluated by the number of survived animals.

Table 3. Impact of Imunofan on immunogenicity of antirabies vaccine

Animal group	Vaccine dilution	% of survived animals
Cultural antirabies vaccine + Imunofan	н/р	85
	1/4	46
	1/16	67
	1/32	71
	1/64	79
Cultural antirabies vaccine	н/р	91
	1/4	79
	1/16	79
	1/32	57
	1/64	46
Imunofan	-	80

As the Table 3 shows, stimulating effect of Imunofan on immunogenicity of antirabies vaccine in cases of rabies takes place upon the use of vaccine in dilution of 1/32, 1/64, i.e. injection of small dose of vaccine. It should be noted that injection of just Imunofan causes protective effect for 80% animals and it is just by 5-11% lower than protective activity of undiluted vaccine.

Thus, trial with mice demonstrated stimulating effect of Imunofan on immunogenicity of antirabies vaccine and its ability to exert protective activity upon rabies virus inoculation [15,28].

Experimental study of Imunofan impact on immunogenic activity of vaccine against hepatitis A

Trial was conducted with the use of outbread cavy with 300-350 g body weight. Each group included 10 cavy [28]. Experimental vaccine against hepatitis A (cultural, concentrated, purified, inactivated, fluidus) - Hep-A-in-VAK, series 16 (produced by Research and

Development company NPO "Vector" of Novosibirsk) was used for the trial.

Vaccine was subdermally injected into 3 spots of an animal in the area of rear pads and back with total volume of 0,5 ml. Imunofan in the dose of 1,5 mkg per one cavy was subdermally injected in the volume of 0,5 ml into cavy's back area. Vaccine and Imunofan were injected together, 3 times with two-week stretch. Immune strength against hepatitis A was evaluated by specific serum antibody titer (AT) towards hepatitis A virus (VHA) and by seropositive animal number. Heart blood sampling was done twice in immunization time course on the Day 14 after second and third injections. Anti-VHA-antibody titer was estimated in serum samples by diagnosticum "IFA-anti VHA" (developed by Research and Development company "Diagnostic systems" of Nijniy Novgorod).

Table 4. Impact of Imunofan on immunogenic activity of hepatitis A vaccine.

Animal group	Period of blood samplin g	Geometric mean AT titer	AT titer	Rate of seropositive animals %
HepA-in-VAK + Imunofan	I II	1.93?0,30 2,97?0,25	1:85 1:933	90 100
HepA-in-VAK (control)	I II	1 ,40?0,34 2,24?0,39	1:25 1:174	70 90

As the Table 4 displays, after two vaccine injections (1-st period of sampling) group of cavy received vaccine together with Imunofan (trial group) showed AT titers 3,4-fold higher than control group (1:85, 1:25 accordingly). After 3-time injections of medications AT level in the group of Imunofan was found 5,36-fold higher than in the group of vaccine only (1:933. 1:174, accordingly).

Rate of seropositive animals (AT titer higher 1:10) in control group was estimated 70% (1^{st} period of sampling) and 90% (2^{nd} period of sampling), and in trial group it was estimated 90% (1^{st} period of sampling) and 100% (2^{nd} period of sampling).

Thus, trial with cavy demostrated stimulating effect of Imunofan on immunogenicity of hepatitis A vaccine.

Thus, the results of experimental study of peptide immuno-regulatory drug Imunofan have demonstrated ability of the drug to improve immune response upon injection of vaccine with reduced antigen content, improve protective quality of vaccine and increase animal survival rate after virus inoculation.

To evaluate the efficiency of peptide immuno-regulatory drug use compared to therapeutic vaccine activity, clinical-immunologic study of Imunofan administration in patients with brucellosis was conducted. This pathology was picked out due to the fact that brucellosis therapeutic vaccine has broad use to induce specific antibodies for treatment of the disease. Along with that, administration of therapeutic brucellosis vaccine causes a number of side-effects related to reactogenecity of vaccine and increase of allergization among patients. For example, an increase of specific antibody titers among main classes of immunoglobulins IgM, IgG и IgA after vaccine injection also induces significant increase of IgE class antibody titers. Administration of Imunofan by brucellosis patients provides stimulation of specific antibodies of all main classes IgM, IgG, IgA approximately at the same levels as with therapeutic brucellosis vaccine injection. However the level of reagin IgE class antibody production upon administration of Imunofan was almost 2.5-fold lower compared to the effect of therapeutic brucellosis vaccine injection (diagram 3). At the same time Imunofan administration induced decrease of IgE serum levels in patients with initially high levels of serum IgE.

Thus, administration of therapeutic immuno-correcting agent by patients with brucellosis provides certain advantages compared to therapeutic vaccine activity and allows to prevent side-effects, increase of IgE class reagin antibody level and further allergization of an organism.

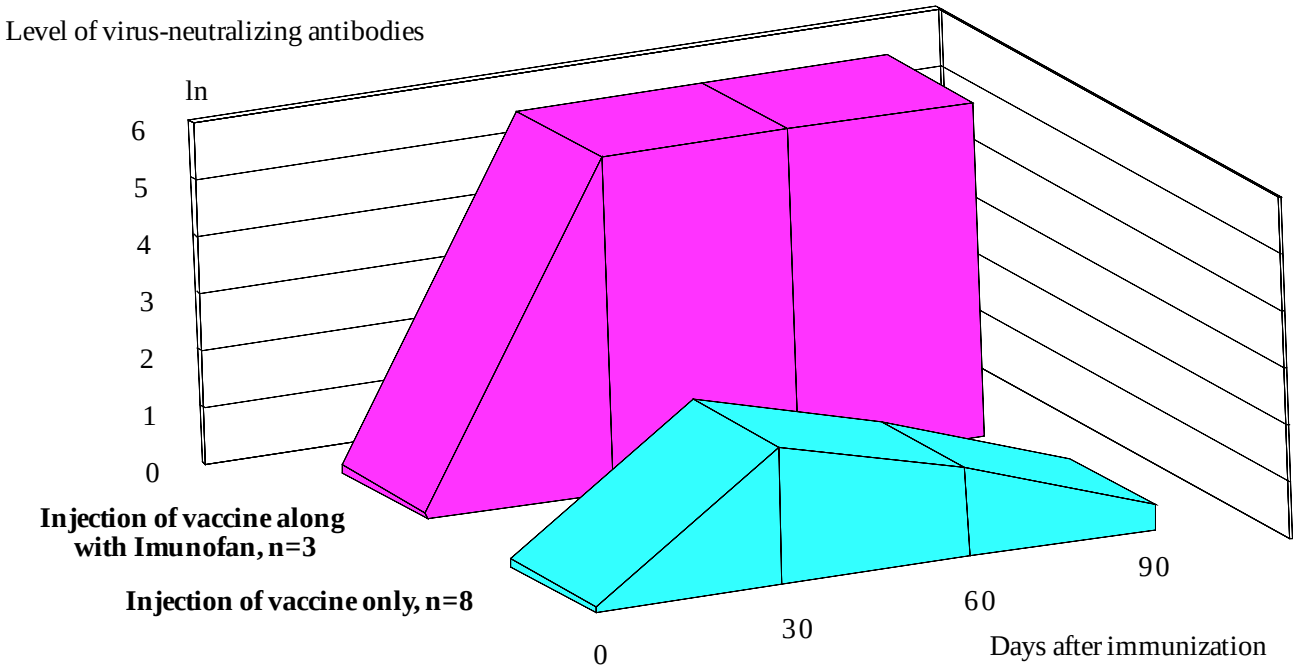
Demonstrated results became the reason for further study of efficacy of immuno-modulating agent use with the purpose of indication extension and safety improvement of vaccine for children with severe allergic pathology. During the period of Imunofan clinical study 100 children of ages from 3 months to 7 years with different allergic conditions were observed. Trial group of children included 20 subjects with bronchial asthma, 30 subjects with atopic dermatitis, 10 children suffered with reccurent urticaria fever and Quincke's edema, 40 subjects with pollinosis. Immunological check-up of the trial group subjects revealed

an increase of IgE plasma levels up to 200-5000 ME/ml. Therapy for the children included intramuscular injections of Imunofan one time a day. Children under 1 year of age were injected 0,5 ml of drug, children over 1 year of age - 1,0 ml. Duration of therapy and number of administered injections were determined individually and varied between 5 and 10 doses. Upon the therapy with Imunofan it was noted that the rate of relapses and clinical symptoms of bronchial asthma decreased, pollinosis and symptoms of postprimary skin infection in patients with atopic dermatitis decreased as well. Improvement of clinical picture with Imunofan therapy was usually noted along with 2-3-fold IgE level decrease compared to initial level (diagram 4).

Right after the end of Imunofan therapy course and IgE level decrease the subjects were injected ADS-m anatoxin vaccine with reduced antigen content. Significant hyperthermic reactions, immediate allergic reactions or relapses of main allergic pathologies in children during post-vaccinal period have not been noted. According to results of diphtheritic antibody evaluation in passive hemagglutination test (RPGA) after 2 months since vaccination, development of healthy immune response was noted.

Thus, regulatory peptide use for children with significant or severe allergic pathology who are subjects for vaccine suspension, makes it possible to decrease hyperproduction of IgE, reduce manifistation of clinical symptoms of pathology, improve vaccine safety qualities and perform vaccination in the needed extent.

Diagram 2. Impact of Imunofan on titer levels and time-duration of virus-neutralizing antibodies circulation upon immunization with infectious virus rhinotracheitis - infectious pustular vulvovaginitis vaccine. Axis of abscissa: days after immunization; axis of ordinates: In of virus-neutralizing antibody titer.



Note: n — number of infected subjects

Diagram 2: Impact of Imunofan on titer values and term of virus-neutralizing antibody circulation upon immunization with viral rhinotracheitis - infectious pustular vulvovaginitis vaccine.

Diagram 3

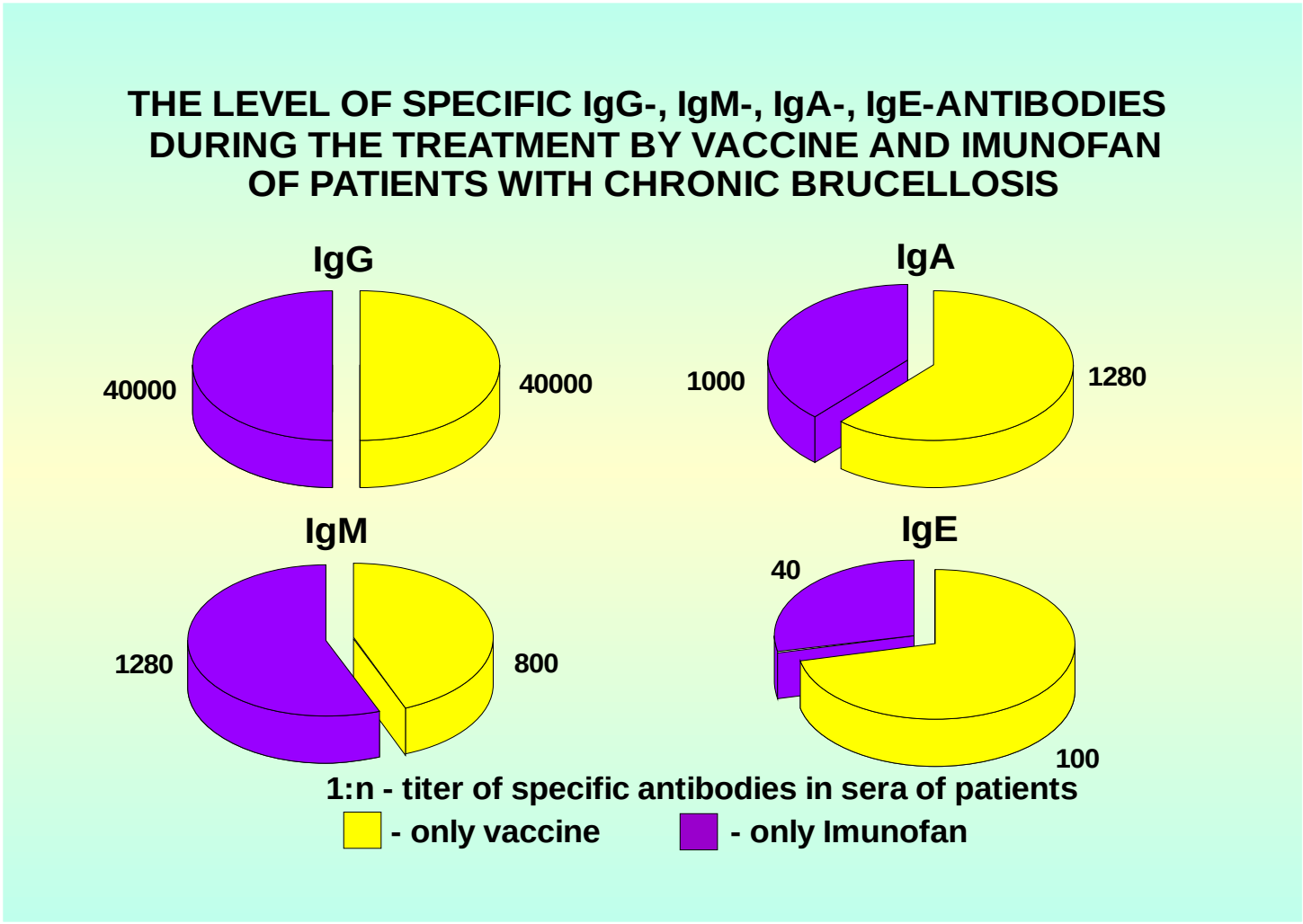

THE LEVEL OF SPECIFIC IgG-, IgM-, IgA-, IgE-ANTIBODIES DURING THE TREATMENT BY VACCINE AND IMUNOFAN OF PATIENTS WITH CHRONIC BRUCELLOSIS
IgG
40000
40000
IgA
1000
1280
IgM
1280
800
IgE
40
100
1:n - titer of specific antibodies in sera of patients
- only vaccine
- only Imunofan

Diagram 4. Influence of Imunofan on the Level of IgE in Serum of Patients with Bronchial Asthma, Atopic Dermatitis, Pollinosis, Angioedema.